DIAGNOSIS AND TREATMENT OF EARLY PRE-TYPE-1 DIABETES

UTILIZING GLIAL FIBRILLARY ACIDIC PROTEIN

FIELD OF THE INVENTION

This invention relates to autoimmune (Type 1A) diabetes mellitus (T1D). Specifically, the invention relates to the early diagnosis of pre-Type-1 Diabetes based on the discovery that Schwann cell proteins, in particular glial fibrillary acidic protein (GFAP) plays a role in early stage autoimmunity, particularly serving as a marker of this process; and most particularly serving for the detection of GFAP binding proteins as the earliest harbingers of future disease risk and providing an unexpected, new target for intervention treatments.

BACKGROUND OF THE INVENTION

T1D in humans and its premier animal model, the non-obese diabetic (NOD) mouse, are polygenic autoimmune diseases whose penetrance is under control of environmental factors (M. Knip, H. K. Akerblom, *Exp Clin Endocrinol Diabetes* 107, S93-100 (1999); D. B. Schranz, A. Lernmark, *Diabetes Metab Rev* 14, 3-29 (1998); G. T. Nepom, W. W. Kwok, *Diabetes* 47, 1177-84 (1998); J. A. Todd, *Pathol Biol (Paris)* 45, 219-27 (1997); M. A. McAleer *et al.*, *Diabetes* 44, 1186-1195 (1995)).

1 Insulin deficiency is the end result of a slowly progressive

2 process, prediabetes, characterized by the accumulation of

3 more and more dense T cell infiltrates around ('peri-

4 insulitis') and eventually inside the islet ('invasive

5 insulitis').

6 This slow progression and its biological controls are

7 not well understood. Without ready access to the sparsely

8 distributed islets in the human pancreas, most concepts of

9 prediabetes progression derive from the rodent models of the

10 disease (A. A. Rossini, E. S. Handler, J. P. Mordes, D. L.

11 Greiner, *Clin Immunol Immunopathol* 74, 2-9 (1995); M. A.

12 Atkinson, E. H. Leiter, *Nat Med* 5, 601-4 (1999)). However,

13 there is strong consensus that human T1D is also

14 characterized by the development of T cells and

15 autoantibodies that recognize β-cell constituents, the former

16 are effectors of β-cell demise during a decade or more of

17 clinically silent prediabetes.

18 Early NOD prediabetes has successfully been targeted by

19 multiple immunotherapies that slow or altogether halt its

20 progression to overt insulin deficiency and thus diabetes (M.

21 A. Atkinson, E. H. Leiter, *Nat Med* 5, 601-4 (1999); S. Winer

22 *et al.*, *J Immunol* **165**, 4086-4094 (2000); D. L. Kaufman *et*

23 *al.*, *Nature* **366**, 69-72 (1993); R. Tisch *et al.*, *Nature* **366**,

24 72-75 (1993); J. Tian *et al.*, *Nature Med.* **2**, 1348-1353

1 (1996); J. Tian *et al.*, *J Exp Med* **183**, 1561-7 (1996); J.

2 Tian, C. Chau, D. L. Kaufman, *Diabetologia* **41**, 237-40 (1998);

3 R. Tisch, R. S. Liblau, X. D. Yang, P. Liblau, H. O.

4 McDevitt, *Diabetes* **47**, 894-9 (1998); R. Tisch *et al.*, *J*

5 *Immunol* **166**, 2122-2132 (2001); J. F. Elliott *et al.*, *Diabetes*

6 **43**, 1494-1499 (1994)). These immunotherapies have all

7 targeted specific autoimmune responses as measured by

8 autoantibodies. The therapeutic effects of the particular

9 autoantigens or relevant epitope peptide fragments from these

10 molecules, derive from the route of application (usually

11 systemically rather than locally), with mechanisms of pre-

12 diabetes delay or cessation ascribed to clonal deletion,

13 anergy induction and modifications of disease-associated

14 cytokine bias. Unfortunately, the autoantibody responses

15 targeted by these immunotherapies appear relatively late in

16 prediabetes (R. B. Lipton *et al.*, *Amer J Epidemiol* **136**, 503-

17 12 (1992); R. B. Lipton *et al.*, *Diabet Med* **9**, 224-32 (1992)),

18 treatments are effective only if applied earlier in

19 prediabetes, while later treatments can precipitate overt

20 disease (K. Bellmann, H. Kolb, S. Rastegar, P. Jee, F. W.

21 Scott, *Diabetologia* **41**, 844-847 (1998); R. Tisch, B. Wang, D.

22 V. Serreze, *J Immunol* **163**, 1178-1187 (1999); S. Winer *et al.*,

23 *J Immunol* **165**, 4086-4094 (2000)).

24   Nevertheless, these observations have engendered

1 optimism in the field that organ-selective autoimmune

2 diseases such as T1D can be successfully prevented in humans

3 at risk for the disease, by immunological interventions that

4 modify the progression of early disease stages. In this, the

5 pressing need for earlier diagnosis of diabetes risk is

6 clear. The present invention represents the by far earliest

7 T1D risk marker identified, and it entails a new therapeutic

8 strategy for early intervention therapy.

9   In the United States, these developments and needs have

10 been acknowledged by considerable increases in funding for

11 diabetes research, including the development of NIH-

12 sponsored, $300 million research efforts such as THE IMMUNE

13 TOLERANCE NETWORK, TRIGR and TRIALNET. These efforts are

14 aimed at unifying strategies for the translation of animal

15 data to human clinical intervention/prevention trials in

16 organ-selective autoimmune diseases, with T1D the leading

17 concern – reflecting its 100+ billion dollar annual cost in

18 the US (~80% of the total diabetes burden).

19   The past two decades of human T1D research had as its

20 main theme the development of techniques that would allow

21 reliable detection of prodromal disease states and pre-

22 diabetes (W. Karges, *et al.*, *Molec Aspects Med* **16**, 79-213

23 (1995); D. B. Schranz, A. Lernmark, *Diabetes Metab Rev* **14**, 3-

24 29 (1998); R. B. Lipton *et al.*, *Amer J Epidemiol* **136**, 503-12

1 (1992); R. B. Lipton *et al.*, *Diabet Med* **9**, 224-32 (1992); C.

2 F. Verge *et al.*, *Diabetes* **45**, 926-33 (1996); W. Woo *et al.*, *J*

3 *Immunol Methods* **244**, 91-103. (2000)).

4   International workshops continue to provide important

5 controls and improvements in these diagnostic efforts (C. F.

6 Verge *et al.*, *Diabetes* **47**, 1857-66 (1998); R. S. Schmidli, P.

7 G. Colman, E. Bonifacio, and Participating Laboratories,

8 *Diabetes* **44**, 631-635 (1995); R. S. Schmidli, P. G. Colman, E.

9 Bonifacio, G. F. Bottazzo, L. C. Harrison, *Diabetes* **43**, 1005-

10 9 (1994);N. K. MacLaren, K. Lafferty, *Diabetes* **42**, 1099-1104

11 (1993)). However, while the accuracy of pre-diabetes

12 diagnostics is now approaching 90%, it is clear that present

13 autoimmune serology detects only the mid- to late stages of

14 the process with confidence. These stages are characterized

15 in animal models as largely resistant to intervention, and

16 immunotherapy at these stages can accelerate progression and

17 precipitate overt disease (reviewed in S. Winer *et al.*, *J*

18 *Immunol* **165**, 4086-4094 (2000)).

19   Thus the need for very early detection of T1D-risk and

20 impending prediabetes is pressing. While most current

21 studies focus on families with the disease, such techniques

22 must eventually be applicable to the general population,

23 since 85% of new patients do not have a family history of

24 autoimmune disease (W. Karges, J. Ilonen, B. H. Robinson, H.-

1 M. Dosch, *Molec Aspects Med* **16**, 79-213 (1995).

2   It is clear that if a marker indicative of the earliest

3 stages of pre-diabetes could be targeted, that a better

4 understanding and staging of early prediabetes would be

5 realized, and that therapeutic strategies and avenues capable

6 of altering the course, progression and/or manifestation of

7 the disease would be realized. Such a marker of early

8 prediabetes is of paramount importance and is probably a

9 prerequisite for successful human intervention trials.

10

11 SUMMARY OF THE INVENTION

12

13   The above conclusion has re-kindled intense studies of

14 prodromal autoimmunity in animal models. Recent studies by

15 Toronto researchers have added a new concept in these

16 efforts. Thus Winer et al reported that T1D and multiple

17 sclerosis (MS) share a near identical set of

18 autoreactivities, including islet reactive T cells in MS and

19 nervous system autoreactivity in T1D (*J Immunol* **166**, 2832-

20 2841, ibid 4751-4756 (2001)). SYN-X Pharma, Inc. of

21 Mississauga, Ontario has developed proteomics approaches to

22 nervous system diseases including MS, with the discovery of

23 new biomarker molecules for these disease processes though

24 the use of modern mass spectrometry instrumentation. This

25 technology was used to search for disease markers common to

1 both diseases. In this ongoing process, SYN-X scientists

2 discovered a diabetes-associated 150 kD molecule that reacted

3 with nervous system tissue in pancreas and was identified as

4 autoantibody to glial fibrillary acidic protein (GFAP) a

5 component of the Schwann cell mantle surrounding the

6 pancreatic islets of Langerhans (S. R. Donev, *Cell Tissue Res*

7 **237**, 343-8 (1984)). These antibodies appear in female NOD-

8 strain mice as early as 4 weeks of age and are absent in male

9 NOD animals. Female NOD mice develop T1D at a high rate

10 (~90%), while male NOD mice rarely develop the disease. GFAP

11 autoantibodies represent the first identified marker of early

12 pre-diabetes to date, and they imply that peri-islet Schwann

13 cells, i.e. a nervous system tissue, is an unexpected, early

14 target of pre-diabetic autoimmunity.

15 Subsequent studies discovered the presence of similar

16 autoantibodies in patients with diabetes and in relatives

17 with high risk to develop the disease. The appearance of

18 these autoantibodies thus provides a long elusive screening

19 tool for the identification of early, progressive

20 prediabetes, identifying candidates for intervention trials.

21 Given the clear precedence of the ability of using

22 autoantibody targets for immunotherapy (see above)(A.

23 Atkinson, E. H. Leiter, *Nat Med* 5, 601-4 (1999)), it is

24 proposed to target the autoimmune response to GFAP by

1 immunotherapies aimed at modifying the response and halting

2 autoimmune progression. Thus, any therapeutic modality which

3 interferes, e.g. by interference is meant a modality having

4 the ability to in some way alter the course, progression

5 and/or manifestation of the disease, as a result of

6 interfering with the disease manifestation process at the

7 early stages focused upon by the identification of the

8 autoimmune disease (e.g. prediabetes) indicative markers as

9 instantly disclosed, are a part of this invention. Since the

10 underlying autoimmunity in T1D and MS are fundamentally the

11 same (S. Winer *et al.*, *J Immunol* **166**, 2832-2841 (2001); S.

12 Winer *et al.*, *J Immunol* **166**, 4751-4756 (2001)), it is evident

13 that the same arguments and reasoning should apply to both

14 diseases. Thus, it is suggested that at least several organ

15 selective autoimmune diseases are inherently and initially

16 directed towards nervous system components, with disparate

17 tissue factors and elements such as host histocompatibility

18 molecules determining the clinical outcome. This present

19 filing focuses on T1D and MS where relevant similarities have

20 been worked out and reported in the literature.

21 Accordingly, it is an objective of the instant invention

22 to teach a binding protein indicative of a loss of self

23 tolerance of the Schwann cell protein, GFAP, and other SC

24 constituents such as S100_in mammals, notably humans. (S.

1 Schmidt *et al.*, *Brain* (1997); M. Popovic, J. Sketelj, M.

2 Bresjanac, *Pflugers Arch* **431**, R287-8 (1996))., which will be

3 referred to as "SC autoantibodies" and will include all

4 immunologically detectable fragments thereof.

5   It is a further objective of the instant invention to

6 teach a method and a device for the use of SC autoantibodies

7 as a predictive marker of organ selective autoimmune disease

8 such as T1D and MS, either in the format of a point-of-care

9 assay or in the format of a central laboratory diagnostic

10 assay.

11   It is yet another objective of the instant invention to

12 provide a diagnostic assay test kit for SC related autoimmune

13 disease, notably for prediabetes and pre-MS.

14   It is a still further objective of the invention to

15 provide a diagnostic assay test kit for prediabetes wherein

16 the SC autoantibody is an anti-GFAP autoantibody supplied in

17 a diagnostically effective amount and the test kit is capable

18 of detecting binding of said diagnostically effective amount

19 of anti-GFAP IgG with a patient sample.

20   It is yet another objective of the instant invention to

21 teach therapeutic targets, therapeutic avenues and

22 therapeutic modalities, along with methods for their

23 determination, isolation and elucidation, which are

24 characterized by their capability for interfering with the

1   course, progression and/or manifestation of the disease, as a

2   result of interfering with the disease manifestation process,

3   for example at the early stages focused upon by the

4   identification of the autoimmune disease (e.g. prediabetes)

5   indicative markers as instantly disclosed.

6   Other objects and advantages of this invention will

7   become apparent from the following description taken in

8   conjunction with the accompanying drawings wherein are set

9   forth, by way of illustration and example, certain

10   embodiments of this invention. The drawings constitute a

11   part of this specification and include exemplary embodiments

12   of the present invention and illustrate various objects and

13   features thereof.

14

15   BRIEF DESCRIPTION OF THE FIGURES

16   Figure 1 illustrates a SELDI process using GFAP-coupled

17   chip arrays;

18   Figure 2 illustrates the presence of GFAP binding

19   protein in 4 week old NOD female mice;

20   Figure 3 illustrates a comparison of male vs. female NOD

21   mice at 5 weeks;

22   Figure 4 (4A, 4B, 4C and 4D) illustrates a comparison of

23   serum samples from patients with recent onset T1D (Fig. 4B),

24   from autoantibody-positive first degree relatives with

1 probable prediabetes (Fig. 4A) and from relatives without

2 signs of autoimmunity (Fig. 4 C, D), which were analyzed in a

3 similar fashion as NOD mice.

4

5 DETAILED DESCRIPTION OF THE INVENTION

6 Since β-cells themselves express trace amounts of GAD65 as

7 well as S100, but lack GFAP expression detectable by RT-PCR,

8 GFAP provides a local SC marker.

9 With reference to Figure 1, IgG autoantibodies to GFAP

10 were measured in sera from NOD mice of different ages, using

11 covalently GFAP-coupled chip arrays in a SELDI-time-of-flight

12 mass spectrometry instrument calibrated with a monoclonal

13 anti-GFAP antibody.

14 As seen in Figure 2, serum from 11/13 NOD females as

15 young as 4 weeks old contained a GFAP-binding protein of

16 149,805.71200 D mass. This 150 kD protein was removed by

17 prior serum passage over solid phase GFAP or solid phase

18 Protein G columns and thus represents IgG autoantibody. These

19 autoantibodies were maintained in overtly diabetic mice 20-26

20 weeks of age. Samples with high autoantibody signals in

21 SELDI-TOF-MS were found to contain anti-GFAP autoantibodies

22 in Western blots, but the sensitivity of SELDI exceeds that

23 of Western blots.

1   As set forth in Figure 3, sera from male NOD mice 5-18

2   weeks of age, from 7 week old non-autoimmune strain C57Bl/6 and

3   8 week old Balb/c mice, or from NOD females 3 weeks of age were

4   negative, while 5/8 samples from 4-5 week old females were

5   clearly positive for GFAP autoantibodies.

6   It was therefore concluded that loss of self-tolerance to

7   the Schwann cell protein, GFAP, and likely other SC

8   constituents such as S100, is a characteristic of NOD mouse

9   prediabetes and predicts the progressive disease course leading

10   to overt T1D in female mice. There is no presently available

11   serum marker to predict disease risk or overt disease in NOD

12   mice before establishment of invasive insulitis by 10-12 weeks

13   of age (S. Reddy, N. Bibby, R. B. Elliott, *Clin Exp Immunol* **81**,

14   400-5 (1990)): in the case of NOD females GFAP autoantibodies

15   have a positive a predictive power of about 90% at an age of 5

16   weeks, i.e. before insulitis is established. This is an age

17   where intervention therapies have the best effectiveness

18   (discussed in: (S. Winer *et al.*, *J Immunol* **165**, 4086-4094

19   (2000); 1.   M. A. Atkinson, E. H. Leiter, *Nat Med* **5**, 601-4

20   (1999)).

21   Diabetes-associated autoimmunity in NOD mice and humans

22   targets a closely similar set of autoantigens. As seen in

23   Figure 4 (4A, 4B, 4C and 4D) serum samples from patients with

24   recent onset T1D (Fig. 4B), from autoantibody-positive first

1 degree relatives with probable prediabetes (Fig. 4A) and from

2 relatives without signs of autoimmunity (Fig. 4 C, D) were

3 analyzed in a similar fashion as NOD mice. Samples from

4 24/30 new onset patients, 9/10 relatives with probable

5 prediabetes 2/29 healthy controls, and 4/5 patients with

6 probable MS contained anti-GFAP autoantibodies detected by

7 SELDI-TOF-MS.

8   We thus conclude that autoimmunity against peri-insular SC

9 is characteristic of human and NOD mouse T1D and probably MS

10 and thus appears to be a characteristic of the disease in

11 general. Collectively, these observations establish peri-

12 insular SC as a *bona fide* autoimmune target in T1D.

13 Autoantibodies are not thought to be mediators of tissue

14 destruction, but rather reflect the immune system's function to

15 remove detritus once tissue destruction occurred. While it is

16 difficult to rule out subtle β-cell damage this early in the

17 prediabetes process, the first autoantibody and thus the first

18 tissue destruction in prediabetes is the peri-islet SC mantle,

19 i.e. a nervous system tissue. This conclusion provides not only

20 a new diagnostic element in prediabetes, but also an attractive

21 new target for therapeutic, including immunotherapeutic

22 intervention, e.g. modalities such as administration of an

23 immunologically reactive moiety capable of altering the course,

24 progression and/or manifestation of the disease, as a result of

1 interfering with the disease manifestation process at the early

2 stages focused upon by the identification of the disease, e.g.

3 prediabetes indicative markers as instantly disclosed, such as

4 by supplying a moiety capable of modifying the pathogenicity of

5 lymphocytes specific for GFAP or other related SC components.

6 　　　Therapeutic targets may thus be defined as those

7 moieties which are capable of exerting a modulating force,

8 wherein modulation is defined as an alteration in function

9 inclusive of activity, synthesis, production, and circulating

10 levels.　Thus, modulation effects the level or physiological

11 activity of at least one particular disease related

12 biopolymer marker or any compound or biomolecule whose

13 presence, level or activity is linked either directly or

14 indirectly, to an alteration of the presence, level, activity

15 or generic function of the biopolymer marker, and may include

16 pharmaceutical agents, biomolecules that bind to the

17 biopolymer markers, or biomolecules or complexes to which the

18 biopolymer markers bind.　The binding of the biopolymer

19 markers and the therapeutic moiety may result in activation

20 (agonist), inhibition (antagonist), or an increase or

21 decrease in activity or production (modulator) of the

22 biopolymer markers or the bound moiety.　Examples of such

23 therapeutic moieties include, but are not limited to,

24 antibodies, oligonucleotides, proteins (e.g., receptors),

1 RNA, DNA, enzymes, peptides or small molecules.

2   With regard to immunotherapeutic moieties, such a

3 moiety would be an effective analogue for a major epitope

4 peptide in GFAP which reduces the pathogenicity of key

5 lymphocytes which are specific for the native epitope in

6 GFAP. An analogue is defined as having structural similarity

7 but not identity in peptide sequencing able to be recognized

8 by T-cells spontaneously arising and targeting the

9 endogeneous self epitope. A critical function of this

10 analogue is an altered T-cell activation which leads to T-

11 cell anergy or death.

12   As β-cells have gene expression patterns reminiscent of

13 neuronal cells (F. Atouf, P. Czernichow, R. Scharfmann, *J*

14 *Biol Chem* **272**, 1929-34 (1997)), it seems conceivable that

15 interactions between peri-islet SC and intra-islet β-cells

16 have functional interactions typical for peripheral SC and

17 'their' neurons, with the former maintaining the latter. An

18 autoimmune attack on SC would then compromise survival of β-

19 cells and possibly their regeneration. This possible axis of

20 interaction has been uncovered by the observations leading to

21 the present invention and deserve renewed attention as a

22 candidate factor in prediabetes progression: e.g. β-cells

23 may be victims of collateral damage in a primary autoimmune

24 attack on pancreatic nervous system tissue.

1   As used herein the term "marker" or "biopolymer marker"

2   are any molecules, typically proteins that pass out from the

3   organ's cells as the cells become damaged or as adaptation

4   occurs. These proteins can be either in the native form or

5   can be any moiety which contains immunologically detectable

6   or immunologically reactive fragments of the protein,

7   resulting, for example, from proteolytic digestion of the

8   protein. When the terms "marker" "biopolymer marker" or

9   "analyte" are used, they are intended to include fragments

10   thereof that can be immunologically detected. By

11   "immunologically detectable" or "immunologically reactive" is

12   meant that the protein fragments contain an epitope that is

13   specifically recognized by a cognate antibody, e.g. the

14   immunologically reactive marker, moiety or fragment has an

15   affinity for a particular entity, e.g. an antibody.

16   As used herein, the term antibody includes polyclonal

17   and monoclonal antibodies of any isotype (IgA, IgG, IgE, IgD,

18   IgM), or an antigen-binding portion thereof, including but

19   not limited to F(ab) and Fv fragments, single chain

20   antibodies, chimeric antibodies, humanized antibodies, and a

21   Fab expression library.

22   Antibodies useful as detector and capture antibodies in

23   the present invention may be prepared by standard techniques

24   well known in the art. The antibodies can be used in any

1 type of immunoassay. This includes both the two-site

2 sandwich assay and the single site immunoassay of the non-

3 competitive type, as well as in traditional competitive

4 binding assays.

5   Particularly preferred, for ease and simplicity of

6 detection, and its quantitative nature, is the sandwich or

7 double antibody assay of which a number of variations exist,

8 all of which are contemplated by the present invention. For

9 example, in a typical sandwich assay, unlabeled antibody is

10 immobilized on a solid phase, e.g. microtiter plate, and the

11 sample to be tested is added. After a certain period of

12 incubation to allow formation of an antibody-antigen complex,

13 a second antibody, labeled with a reporter molecule capable

14 of inducing a detectable signal, is added and incubation is

15 continued to allow sufficient time for binding with the

16 antigen at a different site, resulting with a formation of a

17 complex of antibody-antigen-labeled antibody. The presence

18 of the antigen is determined by observation of a signal which

19 may be quantitated by comparison with control samples

20 containing known amounts of antigen.

21   The assays may be competitive assays, sandwich assays,

22 and the label may be selected from the group of well-known

23 labels such as radioimmunoassay, fluorescent or

24 chemiluminescence immunoassay, or immunoPCR technology.

1 Extensive discussion of the known immunoassay techniques is

2 not required here since these are known to those of skilled

3 in the art. See Takahashi et al. (Clin Chem 1999;45(8):1307)

4 for S100B assay.

5 Although not wishing to be limited to any particular

6 embodiment, the panel format exemplified herein is known and

7 is commercially available. The panel format is similar to a

8 format currently being used in association with pregnancy

9 testing and is commercially available under the trade-mark

10 BIOSIGN. Any assay device or method in accordance with the

11 objectives of the instant invention is contemplated for use

12 with one or more bodily fluids, said bodily fluids being

13 selected from the group consisting of blood, blood

14 components, urine, saliva, lymph and cerebrospinal fluid.

15 All patents and publications mentioned in this

16 specification are indicative of the levels of those skilled

17 in the art to which the invention pertains. All patents and

18 publications are herein incorporated by reference to the same

19 extent as if each individual publication was specifically and

20 individually indicated to be incorporated by reference.

21 It is to be understood that while a certain form of the

22 invention is illustrated, it is not to be limited to the

23 specific form or arrangement herein described and shown. It

24 will be apparent to those skilled in the art that various

1 changes may be made without departing from the scope of the

2 invention and the invention is not to be considered limited

3 to what is shown and described in the specification.   One

4 skilled in the art will readily appreciate that the present

5 invention is well adapted to carry out the objectives and

6 obtain the ends and advantages mentioned, as well as those

7 inherent therein. The various biomolecules, e.g. antibodies,

8 markers, oligonucleotides, peptides, polypeptides,

9 biologically related compounds, methods, procedures and

10 techniques described herein are presently representative of

11 the preferred embodiments, are intended to be exemplary and

12 are not intended as limitations on the scope. Changes therein

13 and other uses will occur to those skilled in the art which

14 are encompassed within the spirit of the invention and are

15 defined by the scope of the appended claims.   Although the

16 invention has been described in connection with specific

17 preferred embodiments, it should be understood that the

18 invention as claimed should not be unduly limited to such

19 specific embodiments. Indeed, various modifications of the

20 described modes for carrying out the invention which are

21 obvious to those skilled in the art are intended to be within

22 the scope of the following claims.

23

24